Genetic diversity in *Neospora caninum*

By

SARWAT EKRAM AL-QASSAB

A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

Department of Medical and Molecular Biosciences

Faculty of Science

University of Technology, Sydney

Australia

2009

Certificate

This study was conducted in the Department of Medical and Molecular Biosciences, Faculty of Science, University of Technology Sydney under the supervision of Professor John Ellis and Adjunct Professor Michael Reichel.

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirement for a degree except as fully acknowledged within the text.

I also certify that the thesis has written by me and help that I received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I also certify that the thesis has been written by me with additional help from Prof. John Ellis and Prof. Michael Reichel as acknowledged in individual chapters. Furthermore I certify that all information sources and literatures used are indicated in the thesis.

SARWAT EKRAM AL-QASSAB

Acknowledgment

My deepest gratitude goes to my parents. Their unconditional support and blessings made it possible for me to carry on my studies. My greatest debt of gratitude and appreciation is to my wife Shahnaz, whose help, courage and support are behind my achievement. I appreciate her forbearance and patience during the course of my research. I feel obliged to mention my children Israa, Eman and Mustafa who have shown a great sense of responsibilities, understanding and courage.

I would like to express my respect and sincere gratitude to my supervisor Professor John Ellis, for his expert guidance, constructive criticism and continuous support and encouragement throughout my study. It has been a privilege to work with him. His assistance will always be remembered.

I would like to thank my co-supervisor Professor Michael Reichel for his valuable suggestion and comments throughout the period of my candidature.

Special thanks to Dr Najah Nassif for her technical guidance. Her advices are deeply appreciated.

I would like to thank this University for giving me the opportunity to conduct my study and I also appreciated their financial support.

I would like to thank all researchers mentioned through this thesis for the donation of parasite strains and DNA for conduct of this study.

Many thank to Peter Williams (Kemps Creek farm) for the donation of dog puppies for isolation of *Toxoplama gondii*.

Special thanks to Dr Debra Birch, for her valuable assistance on electron microscopy of *T. gondii*.

I am also grateful to Dr Mary Davey, Professor Peter Windsor, Dr Michael Johnson, Dr Craig Hall, and my fellow colleagues from the lab, Amanda Hudson, Catherine James, Irene Sotirchos, Michael Lees, Robert Walker and Matthew Padula.

Refereed publications arising from this thesis

Al-Qassab, S., Reichel, P. M., Ivens, A., Ellis, J. T. (2009) Genetic diversity detected amongst isolates of *Neospora caninum* and development of a multiplex PCR for typing strains. Molecular and Cellular Probes, **23**: 132-9.

Al-Qassab, S., Reichel, M. P., Hall, G., Johnson, M., Windsor, P. A., Jenkins, D., Su, C., Dubey, J. P., Ellis, J. T. (2009) Isolation of *Toxoplasma gondii* from the brain of a dog in Australia and its biological and molecular characterisations. Veterinary Parasitology, **Available online.**

Al-Qassab, S., Reichel, M. P., Ellis, J. T. (2009) A second generation Multiplex PCR for typing strains of *Neospora caninum* using six DNA targets. Molecular and Cellular Probes, **Available online.**

Papers presented at scientific conferences

Al-Qassab, S., Reichel, M. P., Hall, G., Johnson, M., Windsor, P. A., Jenkins, D., Su, C., Dubey, J. P., Ellis, J. T. (2009) Isolation of *Toxoplasma gondii* from the brain of a dog in Australia and its biological and molecular characterisations. Australian Society of Parasitology Meeting, Adelaide, Australia.

Al-Qassab, S., Reichel, M. P., Ivens, A., Ellis, J. T. (2009) Genetic diversity detected amongst isolates of *Neospora caninum* and development of a multiplex PCR for typing strains. British Society of Parasitology, Edinburgh, Scotland.

Abbreviations

ITS1	internal transcribed spacer sequences 1
nPCR	nested PCR
PBS	phosphate-buffered saline
PBST	phosphate-buffered saline Tween-20
PI	post inoculation
RT	room temperature
ss-rDNA	small subunit ribosomal DNA

Abstract

The apicomplexan parasite *Neospora caninum* has been identified as an important cause of disease in the dairy industry because it causes abortion and neonatal mortality in livestock such as cattle, sheep and goats and also causing neuromuscular disorders and death in dogs.

Biological diversity within the species *N. caninum* isolates has not been studied in detail. Limited differences were reported using several genetic approaches such as sequencing of ribosomal DNA. Mini- and microsatellite DNA markers have recently been reported to represent a valuable tool to study genetic diversity within this parasite. This is the topic of this thesis.

In the present study, mini- and microsatellite DNA markers were used successfully to genotype different strains of *N. caninum* which were isolated from bovine and canine hosts and from different geographical regions of the world. Several mini- and microsatellites were identified that detected differences among different isolates of this parasite. A prototype multiplex PCR assay was developed for strain typing of *N. caninum* based on the length polymorphisms detected amongst three different repetitive markers two- minisatellites and one microsatellite. This method is simple, rapid, highly informative and sensitive for the study of *N. caninum* diversity.

Discrimination amongst isolates can be improved by the incorporation of more loci into a multiplex PCR and so additional DNA markers were sought. The study of genomic DNA resulted in the identification of two additional minisatellites and one microsatellite. A second generation multiplex PCR assay was developed for multilocus strain typing of *N. caninum* based on length polymorphisms of the six satellites. This multiplex included three microsatellites and three minisatellites. This assay provides a higher degree of resolution and provides a valuable tool to study genetic variation among different isolates of this parasite.

Various uses of multiplex PCR were investigated in the study of *N. caninum*. Isolation of *N. caninum* from a litter of pups was attempted; the bitch was previously identified as seropositive to *N. caninum*. Multiplex PCR of DNA from the serum of the bitch showed a genotype for *N. caninum* not previously reported in Australia. *Toxoplasma gondii* was isolated from the brain of a dog for the first time in Australia. The identity of the parasite was confirmed by PCR, Western blotting, electron microscopy and cat bioassay. Genotyping of the isolate (TgDgAu1) was determined by

10 PCR-RFLP markers and revealed it to be a Type II strain. Westerb blotting demonstrated the presence of IgM antibodies to *T. gondii* suggesting the bitch was probably infected during pregnancy and the *T. gondii* was transmitted to the pups congenitally therefore, this represents the first description of a natural case of congenitally transmission of *T. gondii* in the dog. Unfortunately *N. caninum* was not isolated during these studies.

Table of contents

List of Table

List of Figures